Bioremediation of Crude Oil and Oily Sludge Contamination

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Oil India Ltd.

By

Tata Energy Research Institute
Microbial Biotechnology
Darbari Seth Block
India Habitat Center
Lodi Road, New Delhi - 110 003

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Introduction

Earth's resources are finite and the depletion of these resources has been closely linked to the rise in human activities which has led to the environmental decadence. Conservation of natural resources and the protection of the environment is of utmost concern for the sustainable development and also the legacy that we leave for future generations.

Waste has been a part of human activity from time immemorial and is a necessary evil of all development. Enormous quantities of organic and inorganic compounds are released into the environment each year as the result of human activities. In some cases, these releases are deliberate and well regulated (e.g., industrial emissions) while in other cases they are accidental and largely unavoidable (e.g., chemical spills). Many of these compounds are both toxic and persistent in terrestrial and aquatic environments. Soil and surface and ground water contamination are the result of the accumulation of these toxic compounds in excess of permissible levels.

The cost of restoring the burgeoning global inventory of contaminated ecosystems is virtually incalculable. As a result, government, industry and the public have recognized the need for more cost effective alternatives to traditional physical and chemical methods of contaminant remediation. Biological technologies afford the most effective clean technology route for pollution abatement as they are the most sustainable in nature.

Among the biotechnological interventions bioremediation, the degradation or stabilization of contaminants by microorganisms (e.g., bacteria, fungi, actinomycetes, and cyanobacteria) is a safe, effective, and economic alternative to traditional methods of remediation. Bioremediation can also be used in conjunction with a wide range of physical and chemical technologies. The rate and mechanism of biodegradation of xenobiotics particularly the petroleum hydrocarbons depends on the interrelationship between the degradative activity of the microorganisms and the environmental factors. In natural ecosystems, the organic pollutants frequently occur in mixture with other synthetic and natural organic compounds which effect the rate of degradation.

What is *in situ* bioremediation

Bioremediation is a process that uses naturally-occurring microorganisms to transform harmful substances to nontoxic compounds. There are innumerable strains of microbes under basic categories of bacteria, yeast or fungi, which degrade oily sludge through digestion of harmful chemicals present in oily sludge into simpler, less toxic or non-toxic

substances. Microorganisms, like all living organisms, need nutrients (such as nitrogen, phosphate, and trace metals), carbon and energy to survive. These beneficial microbes break down a wide variety of organic (carbon-containing) compounds found in oily sludge to obtain energy for their growth. Many species of soil bacteria, for example, use petroleum hydrocarbons as a food/energy source, transforming them into harmless substances consisting mainly of carbon dioxide, water and fatty acids. Bioremediation harness this natural process by promoting the growth of microbes that can effectively degrade specific contaminants and convert them to nontoxic by-products. There are two basic types of bioremediation: "Biostimulation" provides nutrients to the indigenous microbial populations. This promotes growth and increases metabolic activity which is used to degrade contaminants. "Bioaugmentation" introduces specific blends of microorganisms into a contaminated environment or into a bioreactor to initiate the bioremediation process.

What are the advantages of Bioremediation?

- An ecologically sound, natural process; existing microorganisms can increase in numbers when a oily sludge (the contaminant) is present. When the contaminant is degraded, the microbial population naturally declines thereby keeping a natural ecological balance .
- The residues from the biological treatment are usually harmless products (such as carbon dioxide, water and fatty acids).
- Instead of merely transferring contaminants from one environmental medium to another (e.g., from water to the air or to land) bioremediation destroys the target chemicals.
- Bioremediation is usually less expensive than other technologies that are often used to clean up hazardous waste.
- Bioremediation can often be accomplished where the problem is located *("in situ")*. This eliminates the need to transfer large quantities of contaminated waste of site, and the potential threats to human health and the environment that can arise during such transportation.

Track Record

A highly efficient consortium of hydrocarbon degrading bacterial system was developed which was first field tested in small plots for *in-situ* bioremediation of crude oil and oily sludge (tank and guard basin sludge) contaminated soil at Mathura Refinery. The success of this *in situ* bioremediation in small plots prompted us to undertake a project on a larger scale at Mathura Refinery.

Experimental site for *in situ* and on site bioremediation in Mathura Refinery was selected near scrap yard area (Fig. 1). A total of 10,000 m^2 land area with around 4.5% (average) hydrocarbon contamination was marked off into two experiment plots i.e. Plot A and Plot B. Approximately 150 tons of oily sludge was spread in Block A of Plot A and then mixed with tractor. Oily sludge was heterogeneously distributed in the soil samples (collected from experimental blocks). After mixing of oily sludge in soil, the level of contamination of oily sludge in soil at zero time (just before experiment was initiated) was determined.

Bacterial consortium (containing seven bacterial strains) was grown in fermenter on minimal salt medium with hexadecane and phenanthrene as carbon and energy source. After 24 hours of growth cultures were harvested and mixed with a carrier material. After mixing of the bacterial consortium with the carrier material (contained 10^{10} cells/g carrier material) filled in sterilized polythene bags and the bags were then sealed (Fig 2). Sealed culture packets were kept in dark at 25°C for 7 days for curing. Cured culture packets were transported to Mathura Refinery and applied in both the experimental plots. Observations were taken at 45, 90, 135 and 180 days after the initiation of the experiment and the level of contamination was analyzed.

The analysis of the contaminated soils clearly indicated that the application of the bacterial consortium resulted in the decrease of hydrocarbon contamination in Block A of Plot A from 13.41% to 2.9% in 180 days -a decrease of 78% (Fig 3). Similarly in the others blocks of Plot A the decrease in the level of contamination was in the range of 66-87% (Fig 3). In the control plot where no bacterial treatment was applied a loss of only 8% was observed due to natural weathering. GC profile of the various fraction of oily sludge indicated that alkanes upto C32 along with the various aromatics like napthalene, acenapthalene, dibenzothiophene (DBT), pyrene and florene were degraded. This suggests that the application of the bacterial system for remediation enhanced the rate of loss of the contamination and thus reduced the persistence of the contamination in the soil and also reduced the possibility of the contaminant leaching into the ground water considerably.

The phenomenal success of the application of the bacterial system for remediation of the oily sludge at Mathura was followed with the application of this concept at Barauni Refinery, where 300 tons of the oily sludge was spread in 4000 m^2 area (Fig 8). After 55 days a decrease of around 85% was observed due the application of the bacterial system as compared to 11% in the control plots. It was also observed that the application of the nutrients alone resulted in the loss of 51% of oily sludge (Fig 9 & 10).

Objective: *In situ* bioremediation of crude oil and tank bottom oily sludge contaminations.

Duration of study: 12 months

Sites for treatment: After detailed discussions with the professional of R&D and Safety & Environment Division of Oil India, Duliajan the following sites have been identified for the treatment with '***Oilzapper***', the microbial system developed at TERI:

1. Buwser unloading point, OCS-3
2. Farmers field
3. Sludge pits in the Tank farm area
4. The pits filled with formation water containing crude oil from the first exploratory well at Bachnatia, Duliajan, Assam

Methodology:

1. Crude oil and oily-sludge contaminated sites such as the OCS-3, the buwser unloading point in which their is a pond of water, measuring about 60 ft X 70ft as well as the nearby land (60 ft X 20 ft) contaminated with crude oil would be selected as the first site for bioremediation. This pond of water has about 2 inches layer of crude oil and the depth of water varies between a few inches to about 45 - 60 inches. The second site would be the field of a farmer which is contaminated due to the leakage of the crude oil because of damage to the pipe.

   Further the 5 crude oil tanks of 10278 KL and 4 small tanks of 1025 KL generates about 700 tons/ year of tank bottom sludge, which at the moment is disposed in small pits in the tank farm area only and could lead to possible contamination of the ground water. A single pit of one of the 10278 KL tank would be treated with the bacterial system '*Oilzapper*', developed at TERI and the level of contamination of TPH in the pit upto a depth of 1 meter would be determined both at the start and end of the treatment.

   The fourth site would be the land area at Bachnatia, Duliajan in which the formation water along with oil pumped out due to drilling of the well is being stored. At this site the formation water has virtually dried up and a crust of oil has been formed.

   All the four sites would be treated with *"Oilzapper"*, the bacterial system developed at TERI.
2. Crude oil and Oily-sludge would be extracted from samples collected at zero time (just before the experiment is initiated) and contamination levels would be quantified.

3. Crude oil and Oily-sludge degrading bacterial consortium would be grown in large scale at Tata Energy Research Institute, New Delhi and bacterial culture would be transported to the experimental site. Bacterial culture would be sprayed with a mixture of nutrients and then mixed thoroughly for uniform distribution of bacterial cells.
4. Samples would be collected from the contaminated sites at regular intervals for microbiological and chemical analysis. The total petroleum hydrocarbon (TPH) degrading microbial population along with the population of the introduced hydrocarbon degrading bacterial system would be enumerated. Simultaneously crude oil/oily-sludge would also be extracted and quantified gravimetrically to determine the rate of degradation. Residual oily-sludge so extracted would be fractionated on silica gel column into saturates, aromatics, NSO and asphaltene. Saturates be analyzed by GC (capillary column) to see which compounds are degraded. Similarly aromatic compound would be analyzed by GC/HPLC.
5. Heavy metals of contaminated samples would be analyzed at zero time (just before the experiment is initiated) and at the end of the experiment (12 months after the experiment is initiated).
6. A training program would also be conducted for the scientists and engineers of Oil India which would cover the following aspects:
    a. Theoretical aspects of bioremediation.
    b. Exposure to laboratory techniques for bioremediation.
    c. Field application of bioremediation.

The training program would be covered in two phases, the first phase would be at Duliajan where the theoretical aspects as well as the field application of bioremediation would be dealt with during the process of treatment of the sites at Oil India, Duliajan. The second phase would be at TERI, Delhi for five days in which the scientists and engineers of Oil India would be acquainted with basic laboratory techniques involved with bioremediation.

BUDGET

Purpose of Grant	Amount (Rs)
1. Professional Time	3,88,000
2. Production of ***'Oilzapper'***, TERI's microbial system	2,00,000
3. Consumables A) Biochemical, Chemicals and Glassware B) Items for equipment (Columns, High purity gases for GC and HPLC, HPLC grade solvents, atomic absorption) c) Maintenance of equipment's	 1,50,000 1,00,000 50,000
4. Travel	1,50,000
5. Training	25,000
Subtotal	**10,63,000**
Overheads @ 10% (On items 2, 3 ,4 and 5 only)	67,500
TOTAL	**11,30,500**

Justification:

A) Biochemical, chemicals and glassware would be required for the extraction and fractionation of crude oil and tank bottom oily-sludge, chemical constituents of soil and heavy metals analysis of soil. Consumable items for equipment would be required for purchase of columns for GC and HPLC, High purity gases, HPLC grade solvents etc.

B) Travel grant would be required for the visit of the professionals to set up the experiment and monitor the experiment at Duliajan at regular intervals.

C) Maintenance grant would be utilized for the annual maintenance contract of equipment's; GC, HPLC, atomic absorption, fermenter and other required for the various analysis of soil, crude oil and tank bottom oily-sludge.

D) The present study is highly labor intensive and three professionals would be engaged to carry out this study at Duliajan and at TERI Delhi.

TERI'S Contribution

A) The professional cost of a senior scientist(part time) as well as one technical assistance would be contributed by the institute.

B) The institute would also provide assess to the professionals the equipment's; GC, HPLC, atomic absorption, spectrophotometer as well as all the other equipment's required for the various analysis of the crude oil, tank bottom oily sludge and soil.

Help from Oil India, Duliajan

- ☐ Five casual labourers would be required (for 5 to 7 days) during set up of the experiment and four casual labourers would be required (for one or two days) at interval of 60 days (Observation will be taken at interval of 60 days).
- ☐ Water source would be required at the experimental site for maintaining the moisture level during the course of experiments.
- ☐ One tractor with a harrow would be required on particular days for preparing the site and mixing of oily-sludge at interval of 60 days.
- ☐ Boarding, lodging and local transport would be borne by oil India during visit to Duliajan.

Table 1 Total petroleum hydrocarbon (%) at different depth of experimental plot A

Blocks	25 cm depth		50 cm depth	
	Zero time	After 360 days	Zero time	After 360 days
A	13.41	0.50	0.03	0.02
B	4.26	0.42	0.03	0.03
C	3.19	0.34	0.02	0.01
D	13.20	11.35	0.02	0.02

Note TPH could not be detected at 75 cm and 100 cm depths

Table 2 Total petroleum hydrocarbon (%) at different depth of experimental plot B

Blocks	25 cm depth		50 cm depth	
	Zero time	After 360 days	Zero time	After 360 days
A	7.52	0.80	0.01	0.01
B	2.97	0.38	0.01	0.01
C	3.33	0.45	0.02	0.02
D	13.20	11.35	0.02	0.02

Note TPH could not be detected at 75 cm and 100 cm depths

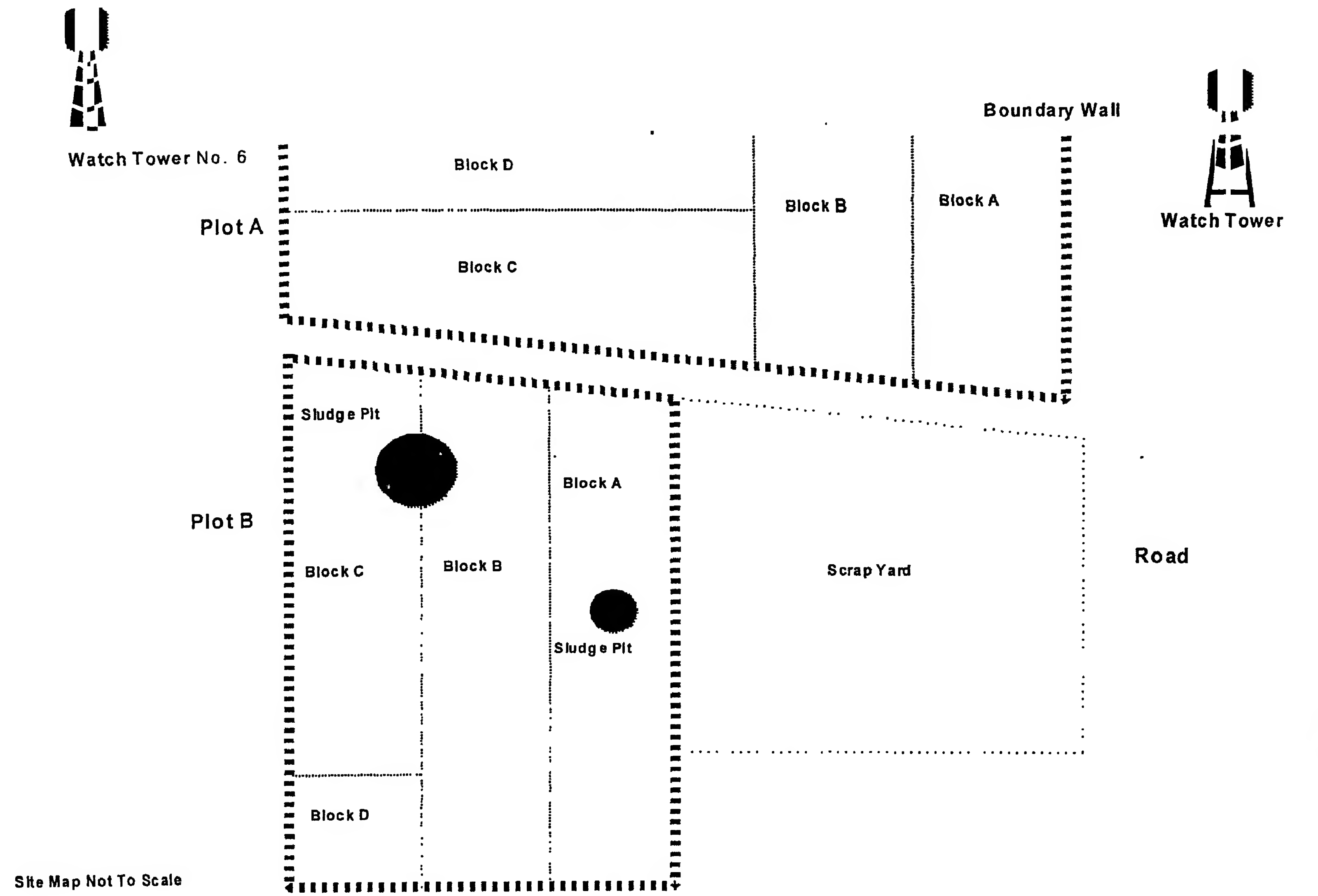

Figure 1: Layout of Experimental Plots at Mathura Refinery

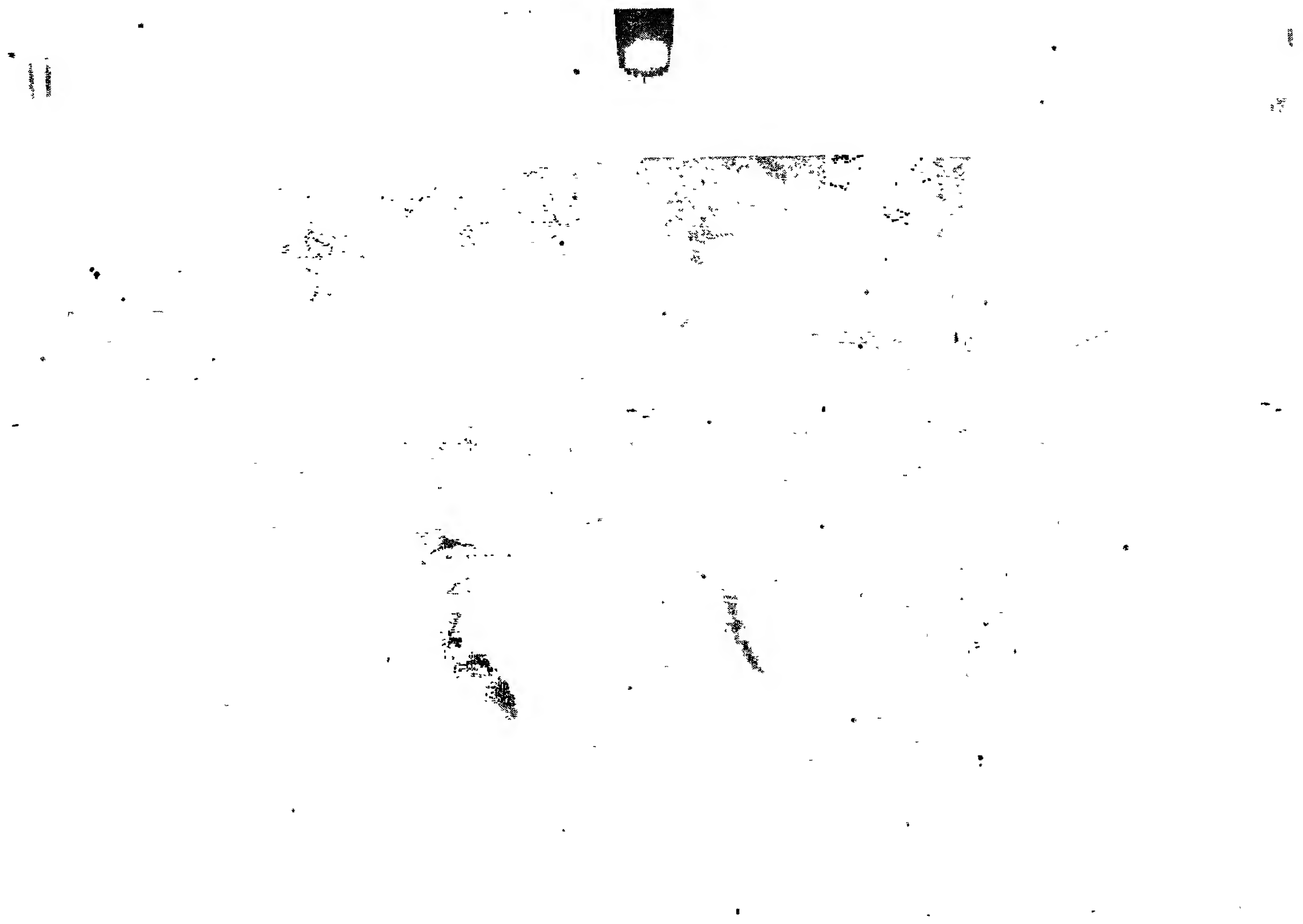

Figure 2: Carrier based crude oil degrading bacterial consortium

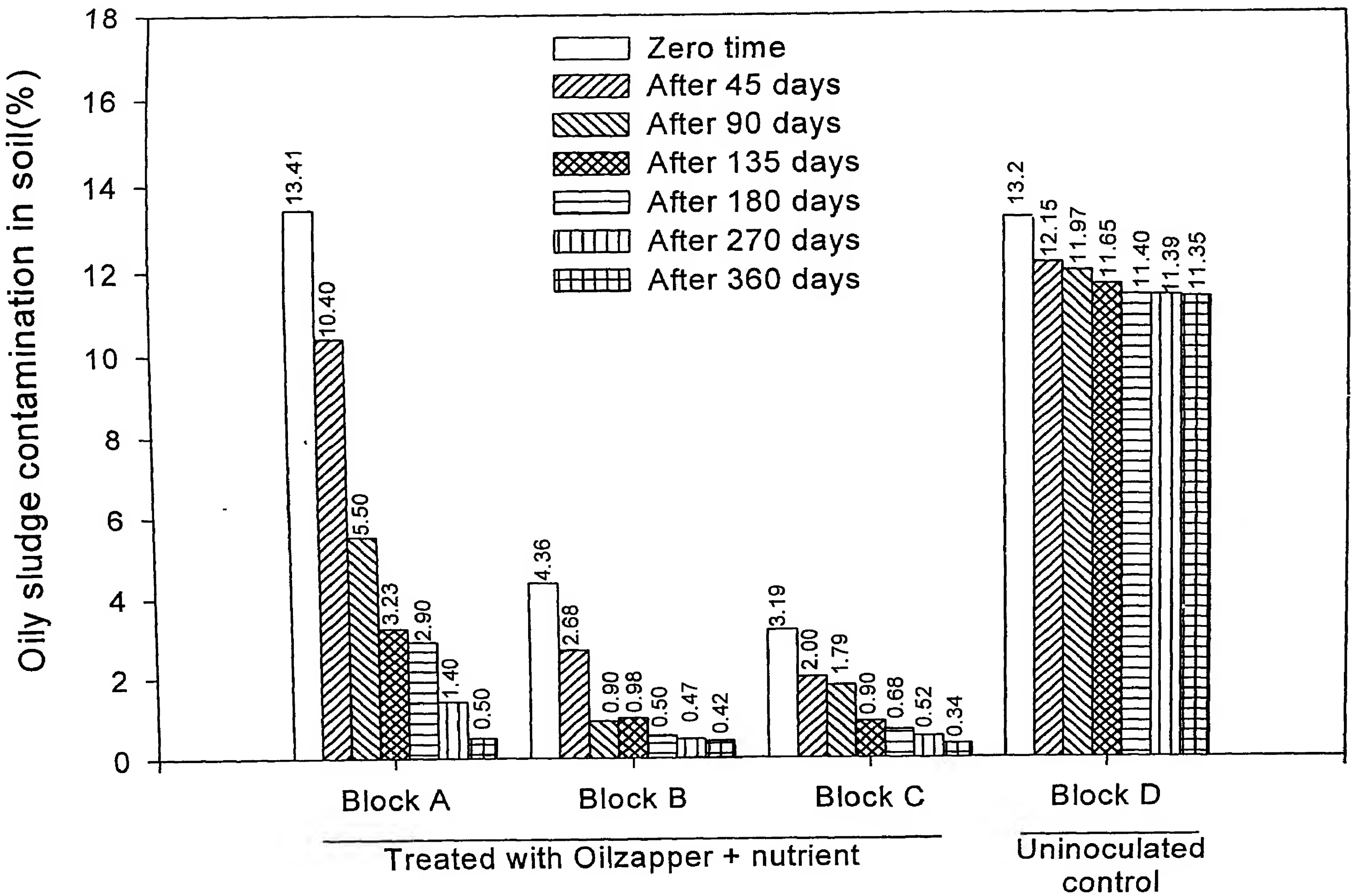

Figure 3 *In-situ* degradation of oily sludge in soil of experimental plot A

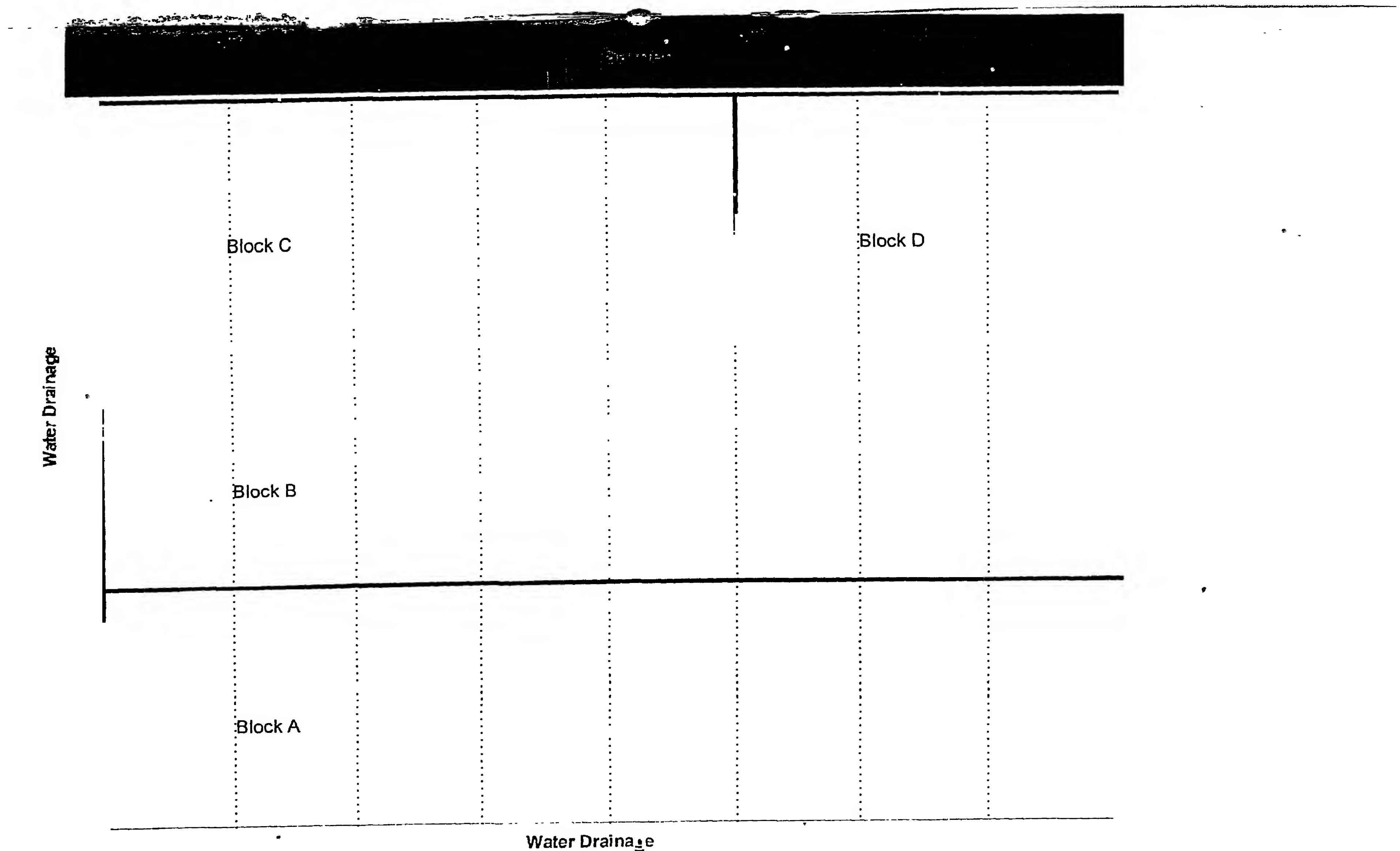

Figure 8: Layout of experimental plot at Barauni Refinery

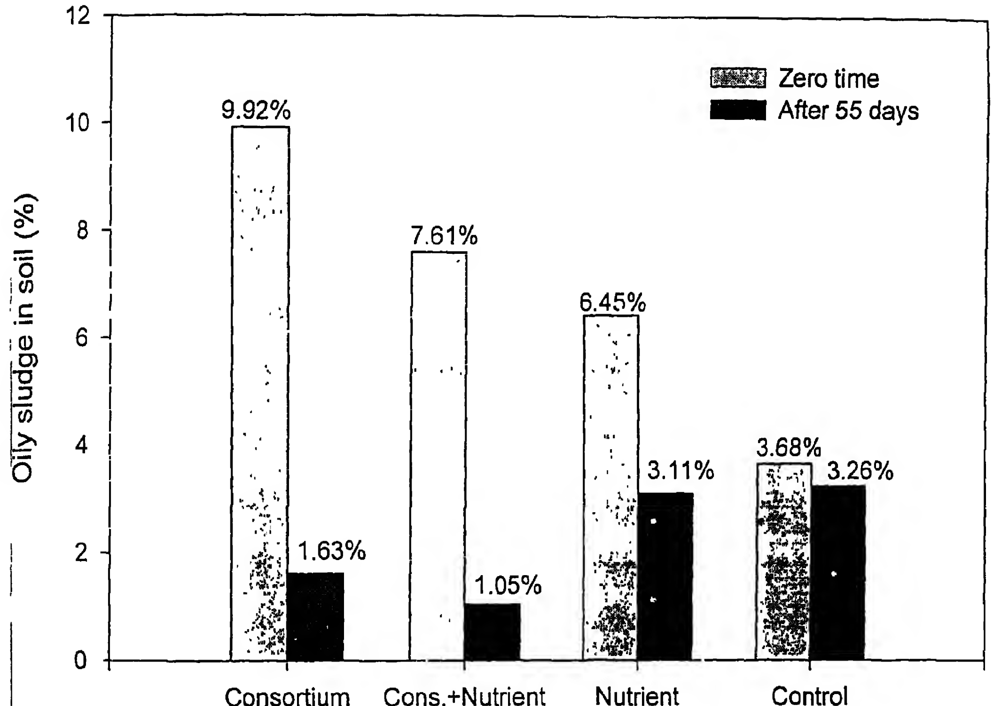

Figure 9: Oily sludge contamination in soil of experimental blocks at Barauni refinery

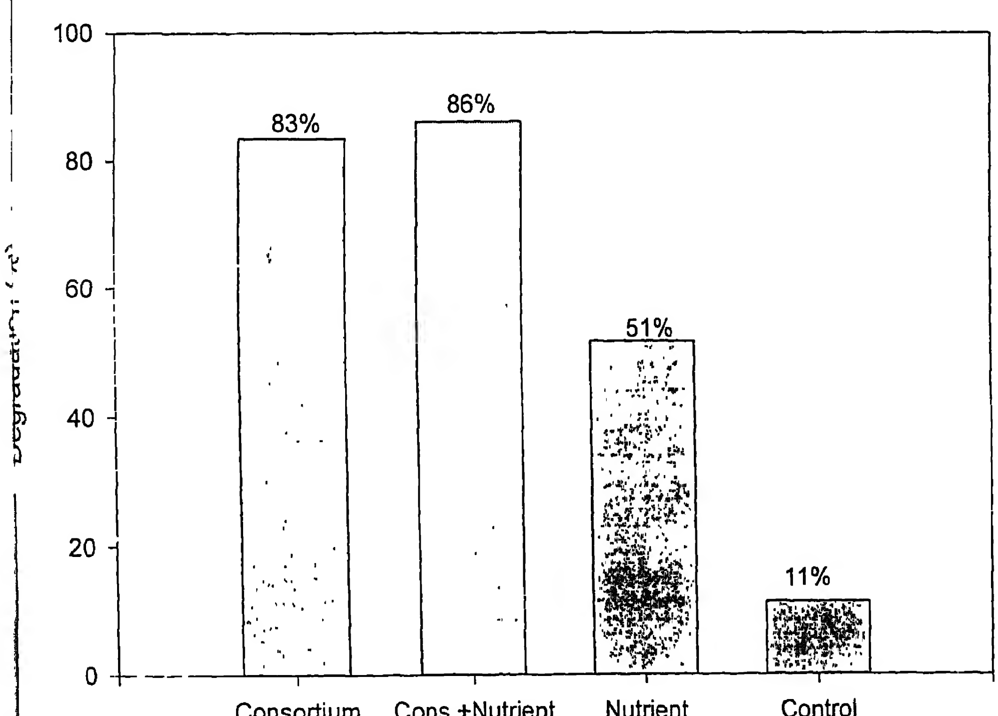

Figure 10: Degradation of oily sludge in experimental blocks at Barauni refinery